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Clinical periodontal status and inflammatory cytokines in gestational diabetes mellitus

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ABSTRACT

Objectives: The aim of the present cross-sectional study was to compare clinical periodontal findings as well as gingival crevicular fluid (GCF) and serum levels of tumour necrosis factor-alpha (TNF-α), interleukin-10 (IL-10), and IL-33 between women with and without gestational diabetes mellitus (GDM).
Methods: Serum and GCF samples were collected, full-mouth recordings comprising plaque index, bleeding on probing and probing depth were performed in 96 females with GDM (cases) and 65 non-diabetic pregnant females (controls). Age, smoking status, pre-pregnancy body mass index, pregnancy outcomes were recorded. Serum and GCF IL-10, IL-33, TNF-α levels were determined.
Results: The GDM group was significantly older than the control group with an age difference of 3.27 years (mean ages were 32.05 and 28.78 years, respectively) ($p < 0.0001$). Plaque Index (50.0 and 30.0 $p = 0.005$), bleeding on probing (50.0 and 30.0 $p = 0.003$) values were significantly higher in the GDM group. Serum TNF-α concentrations were significantly higher in the nonGDM group than the GDM group ($p = 0.001$). GCF IL-10 concentrations and total amounts were significantly higher in the GDM group than the controls ($p = 0.004$ and $p < 0.0001$, respectively).
Conclusion: Elevated GCF IL-10 levels may be a consequence of higher levels of inflammation as indicated by higher PI and BOP in the GDM group. However, the investigated clinical parameters may not have prominent effects on TNF-α and IL-33 levels. These findings provide further support for the importance of periodontal health during pregnancy.

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1. Introduction

Periodontal disease is an inflammatory disease of the tooth-supporting tissues leading to attachment loss, bone loss, and possibly tooth loss if left untreated. Predominantly pathogenic microorganisms colonized in the subgingival area may cause local and systemic elevations of pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α) and interleukin-10 (IL-10), resulting in periodontal tissue destruction. Association between periodontal and systemic diseases including metabolic syndrome, cardiovascular disease and diabetes have been reported (Kinane, Bouchard, Group of European Workshop on Periodontology, 2008).

Gestational diabetes mellitus (GDM) is defined as glucose intolerance with first onset or presentation in pregnancy. There is a progressive insulin resistance during the third trimester of gestation and GDM may develop if pancreas fails to respond with an appropriate increase in ß-cell mass (Rieck & Kaestner, 2010) and insulin secretion (Buchanan, Xiang, Kjos, & Watanabe, 2007). GDM that has a prevalence of 4–10% among pregnant women is associated with significantly increased risks of maternal and infant morbidity such as preeclampsia, preterm birth, macrosomia and also the risk of developing diabetes in later life (ACOG Practice Bulletin, 2001; Xiong, Saunders, Wang, & Demianczuk, 2001; Pridjian & Benjamin, 2010). An estimated 35–60% of women with GDM will develop type 2 diabetes mellitus (DM) within 10 years (Kim, Newton, & Knopp, 2002). Type 2 DM is one of the most common chronic diseases globally (Harris, 1998). Thus, GDM is important for early identification of risk factors and early interventions to prevent later DM development in young females.

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The interleukin-1 (IL-1) family of cytokines is important in destructive inflammatory disorders, such as rheumatoid arthritis and periodontitis (Barksby, Lea, Preshaw, & Taylor, 2007). IL-33 is the most recently discovered member of the IL-1 family (Schmitz et al., 2005). It has been reported that modest levels of IL-33 were detected in lipopolysaccharide-activated human monocytes (Schmitz et al., 2005). Strong evidence exists for a role of IL-33 in regulating T helper type 2 (Th2) cytokines and in stimulating mast cell development and associated pathologies. But the expression and regulation of IL-33 in periodontal tissue cells or biologic fluids have not been clarified yet.

There is immune system modulation during pregnancy (Raghupathy, 1997). IL-10 seems to be a key cytokine that is fundamental to promote normal pregnancy outcomes. Recent findings show a reduction of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), IL-1ß, and IL-6 and an increase of counter regulatory cytokines, such as IL-10 during pregnancy (Brogin Moreli, Cirino Ruocco, Vernini, Rudge, & Calderon, 2012;Taylor, Verhagen, Blaser, Akdis, & Akdis, 2006). On the other hand, pregnancy is a stressful state with increased inflammatory activity (Redman, Sacks, & Sargent, 1999), gingival inflammation (Laine, 2002; Sooriyamoorthy & Gower, 1989), and insulin resistance (Kuhl, 1991; Williams, 2003). Pancreatic ß-cell destruction has been suggested to be related with the pro-inflammatory imbalance created by a sustained elevation of cytokines like IL-1ß and TNF-α (Moller, 2000). High TNF-α κονκεντρατονση αωε βεενρελατεδ with pregnancy complications such as preeclampsia, GDM and preterm birth may also reduce IL-10 levels (Denney et al., 2011; Raghupathy, 2001; Peraçoli, Rudge, & Peraçoli, 2007).

Viable bacteria, bacterial products like lipopolysaccharide from the subgingival plaque as well as pro-inflammatory cytokines like TNF-α, IL-1ß, -6, -8, and C-reactive protein secreted by the inflamed periodontal tissues can enter the circulation to trigger a maternal systemic inflammatory response (Amar & Han, 2003; Garcia, Henshaw, & Krall, 2001). IL-33 is another cytokine activating innate immunity during tissue damage and infection (D'Aiuto et al., 2008; Cayrol & Girard, 2009). Current evidence shows that DM and persisting hyperglycaemia lead to exaggerated immune-inflammatory response to challenges of periodontopathogens, which cause more rapid and severe periodontal tissue destruction (Kingman & Albandar, 2002). On the other hand, infection is known to cause insulin resistance (Agwunobi, Reid, Maycock, Little, & Carlson, 2000). A relationship between periodontal disease and type 2 DM has been suggested (Agwunobi et al., 2000; Chapple, Genco, Working group 2 of joint EFP/AAP workshop, 2013). However, few studies so far have examined a possible relationship between GDM and periodontal disease (Xiong, Buekens, Fraser, Beck, & Offenbacher, 2006; Xiong, Buekens, Vastardis, & Yu, 2007; Xiong et al., 2013; Gümüş et al., 2015).

It is hypothesized that maternal gingival inflammation could induce a systemic inflammatory response causing insulin resistance (Santos Tunes, Foss-Freitas, & Nogueira-Filho Gda, 2010). Such an infection-induced insulin resistance could exacerbate the pre-existing pregnancy-induced insulin resistance and impair glucose tolerance eventually leading to GDM. The aim of the present study was to evaluate the clinical periodontal status as well as gingival crevicular fluid (GCF) and serum levels of TNF-α, IL-10, and IL-33 in women with or without GDM.

2. Materials and methods

2.1. Study population

Ninety-six non-obese females with GDM (aged 22–44 years) and 65 pregnant women without GDM (aged 19–38 years) visiting the Endocrinology and Metabolism outpatient clinic, State Hospital of Aydın, Turkey were recruited for this cross-sectional study between September 2012 and March 2013. The study was conducted in full accordance with ethical principles, including the World Medical Association's Declaration of Helsinki, as revised in 2008. Protocol of the study was approved by the Ethics Committee of Ege University Medical Faculty (Protocol number; 13-2/9). The study protocol was explained to each individual at the beginning of the study and written informed consent was received before enrolment in the study. The study conforms to STROBE guidelines for case-control studies (von Elm et al., 2007). Detailed medical and dental histories were obtained from each individual before any clinical examination and biofluid sampling.

GDM was diagnosed according to the criteria for the diagnosis of diabetes (American Diabetes Association, 2013). Participants who had a 75 g oral glucose tolerance test (OGTT) with plasma glucose measurement fasting and at 1 h, 2 h, at 24–28 weeks of gestation in women not previously diagnosed with overt diabetes were diagnosed as GDM. GDM was based on only one blood glucose value above the specified cut-off points (Fasting: $\geq$92 mg/dL (5.1 mmol/L), 1 h: $\geq$180 mg/dL (10.0 mmol/L), 2 h: $\geq$153 mg/dL (8.5 mmol/L)). Non-obese pregnant women with no known diabetes and not meeting the diagnostic criteria for GDM were included in the control group. Smoking status was determined according to the self-reports and those currently smoking as well as those who used to smoke before pregnancy were excluded from the study.

GCF and serum sampling were described previously (Gümüş et al., 2015). All GCF and serum samples were stored at −40 °C until the laboratory analyses and thawed immediately before assay.

2.2. Clinical periodontal measurements

During the 24–28 weeks of gestation, clinical periodontal measurements including probing depth (PD), plaque index (PI) and bleeding on probing (BOP) were recorded using a periodontal probe (Williams periodontal probe, Hu-Friedy, Chicago, IL) at four sites (mesio-buccal, mid-buccal, disto-buccal, mid-lingual) of each tooth present, except the third molars. BOP was deemed positive if it occurred within 15 s after periodontal probing. Visible plaque accumulation was recorded dichotomously by visual examination. The measurements were conducted by three periodontists who were trained and calibrated before the start of the study and every 6-month during the study (ÖÖ, AA, PG). Weighted κ scores were above 85% and considered nearly perfect (Landis & Koch, 1977). The endocrinologist (BCO) knew whether the pregnant woman had GDM or not and the three periodontists were blinded to the study group during the clinical periodontal measurements.

2.3. Measurement of TNF-α, IL-10 and IL-33 in GCF and serum samples

The pooled GCF samples from the two paper strips from each patient were eluted into 0.5 mL phosphate-buffered saline. Specific enzyme-linked immunosorbent assay (ELISA) kits for IL-10, TNF-α and IL-33 were used for quantitative analyses (eBioscience, San Diego, CA). The assays were performed according to the manufacturer's recommendations. The minimum detection limits were 1.0 pg/mL, 5 pg/mL, and 0.2 pg/mL for IL-10, TNF-α, and IL-33, respectively. Fifty microliter aliquots of GCF or serum were used for all assays and each assay was performed in duplicate.

2.4. Statistical analysis

A previous study, in which IL-33 levels were measured and a 20% difference was obtained, was used for statistical power calculations (Buduneli, Özçaka, & Nalbantsoy, 2012). With a power

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